CLAIMS

1. Method for quantitative and semi-quantitative determination of endogenous amino acids L-phenyalanine, L-tyrosine, L-3,4-dihydroxyphenylalanine and their corresponding keto-acids, phenylpiruvic acid, 3-hydroxyphenylpyruvic acid and 3,4-dihydroxyphenylpyruvic in biological fluids useful for diagnosis and monitoring of metabolic disorders of said amino acids or diseases involving said amino acids, comprising the following steps:

a) reaction of phenylpiruvic acid, 3-hydroxyphenylpyruvic acid and 3,4-dihydroxyphenylpyruvic, present as such in biological fluids or coming from the parent endogenous amino acids L-phenyalanine, L-tyrosine, L-3,4-dihydroxyphenylalanine by deamination, with an organic salt of phenazine derivatives in the presence of at least one alkaline buffer to give colored charge transfer complexes;

b) measurement of the absorbance values due to said charge transfer complexes in the wavelength range from 650 to 690 nm and quantification of the keto acids or amino acids concentrations in biological fluids.

2. Method according to Claim 1, wherein the organic salt of phenazine derivatives is selected from the group consisting of phenazine methosulphate (PMS) or phenazine ethosulphate (PES).

3. Method according to Claim 1, wherein the biological fluids are serum, blood or urine.

4. Method according to Claim 1, wherein the biological fluid is serum and the endogeneous amino acids are previously deaminated by means of a chemical or enzymatic reaction.

5. Method according to Claim 4, wherein the deamination reaction of the endogenous amino acids is catalyzed by the enzyme L-amino acid oxidase in the presence of an alkaline buffer.

6. Method according to Claim 4, wherein, when the endogenous amino acid to be determined is L-phenyalanine, the deamination reaction is performed using the enzyme L-phenyalanine dehydrogenase in alkaline conditions and in the presence of a redox cofactor.

7. Method according to Claim 6, wherein the redox cofactor is NAD^+.

8. Method according to Claim 1, wherein said at least one alkaline buffer is selected from the group consisting of potassium phosphate buffer, sodium phosphate buffer, TRIS-HCl buffer.

9. Method according to Claim 1, wherein step a) is performed in solution or on solid support.

10. Method according to Claim 1, wherein said metabolic disorders or diseases involving L-phenyalanine, L-tyrosine, L-3,4-dihydroxyphenylalanine are Phenylketonuria, Hyperphenylalaninaemia, Tyrosinaemia, Neuroblastoma, Parkinson disease.

11. Kit for diagnosis and monitoring of metabolic disorders of L-phenyalanine, L-tyrosine, L-3,4-dihydroxyphenylalanine or diseases involving said amino acids, based on quantitative detection of the parent keto-acids phenylpiruvic acid, 3-hydroxyphenylpyruvic acid and 3,4-dihydroxyphenylpyruvic comprising:

a) an organic salt of phenazine derivative in solution or adsorbed on solid support;

b) an alkaline buffer such as the extinction coefficients at 663 nm of complexes of phenylpiruvic acid, 3-hydroxyphenylpyruvic acid and 3,4-dihydroxyphenylpyruvic with the organic salt of phenazine derivatives are higher than 13,000 M^{-1} cm^{-1}.

12. Kit according to Claim 11, wherein the organic salt of phenazine derivative is selected from the group consisting of phenazine methosulphate (PMS) or phenazine ethosulphate (PES).

13. Kit according to Claim 11, wherein the solid support is cellulose or equivalent absorbing materials.

14. Kit according to Claim 11, wherein the alkaline buffer b) is potassium phosphate buffer or sodium phosphate buffer.

15. Kit according to Claim 12, which also comprises:

c) a second alkaline buffer which lowers the extinction coefficients at 663 nm of complexes of phenylpiruvic acid, 3-hydroxyphenylpyruvic acid and 3,4-dihydroxyphenylpyruvic with an organic salt of phenazine derivatives below 3,600 M^{-1} cm^{-1}.

16. Kit according to Claim 15, wherein said second alkaline buffer c) is selected from the group consisting of TRIS-HCl buffer, glycine-NaOH buffer, borate buffer.

17. Kit according to anyone of the Claims from 11 to 16, which also comprises:

d) a deaminating enzyme for L-phenyalanine, L-tyrosine, L-3,4-dihydroxyphenylalanine.

18. Kit according to Claim 17, wherein the enzyme is L-amino acid oxidase.

19. Kit according to Claim 17, wherein the enzyme is L-phenylalanine dehydrogenase in the presence of a redox coenzyme when the target amino acid is only L-phenyalanine.

20. Kit according to Claim 19, wherein the redox coenzyme is NAD^+.